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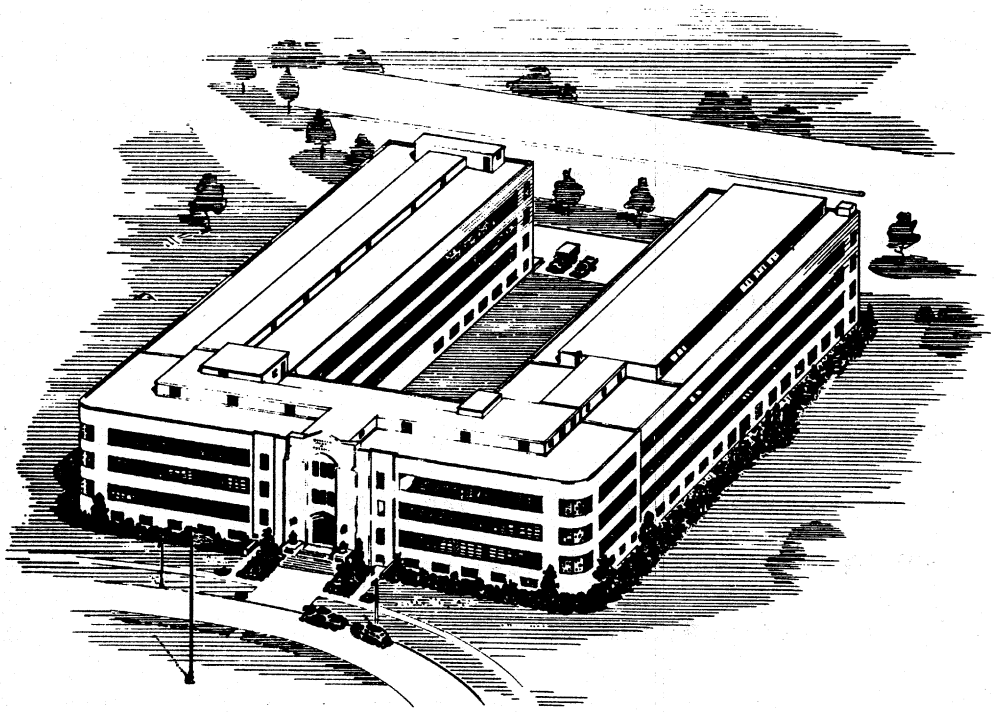

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NICOTIANA RUSTICA. DISTRIBUTION OF NICOTINE IN THE PLANT, LOSS OF NICOTINE DURING DRYING, AND METHODS OF ANALYSES FOR NICOTINE AND MOISTURE[1/]

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INTRODUCTION

Previous investigations of the nicotine content of *Nicotiana rustica* have been concerned primarily with agronomic phases of the problem, such as the effects of fertilizers, varieties, climatic conditions, topping, and suckering on the yield of nicotine per acre (1, 2).

One aspect of the problem has been largely neglected--the possible loss of nicotine caused by drying. In practically all cases, yields of nicotine have been based on the nicotine contents of air-cured plants. If appreciable quantities of nicotine are lost during drying, development of an extraction process for the green plant would be imperative. This would also require the establishment of extraction plants in the growing areas. On the other hand, negligible loss of nicotine during drying would permit the adoption of less costly processes, such as air curing, or rapid drying in existing commercial equipment.

This report gives the results obtained in laboratory drying experiments on green samples grown in 1946, 1947, and 1948. The effects of air curing and of artificial drying on the nicotine content were studied. The loss of nicotine under drastic drying conditions was determined. Improved methods were developed for sampling and analyzing relatively large quantities of green and dried plant material. Finally, the distribution of nicotine in the plant was established.

The varieties used were U. S. D. A. 34753, T. I. 23, and Olson 68. They were grown on experimental plots at Wyndmoor, Pa.

METHODS OF ANALYSIS

C. O. Willits and C. Ricciuti

Determination of nicotine presented three problems: (a) obtaining a representative sample of both the fresh green plants and of the dried product, (b) determination of moisture, and (c) determination of the total nicotine in both these materials on a moisture-free basis. Since the criterion for possible loss of nicotine was to be the difference between the nicotine content before and after drying, it was imperative that the values for total nicotine and moisture have a high degree of accuracy. The dried products offered no particular sampling problem, but this was not the case with the cut segments of fresh green leaves. The poor precision of the nicotine values obtained in the 1946 studies might have been caused by the sampling procedure used. Consequently a new sampling procedure was devised and used throughout the remainder of these studies. Statistical treatment of the data demonstrated that the new sampling technique gave the required precision.

Determination of Nicotine

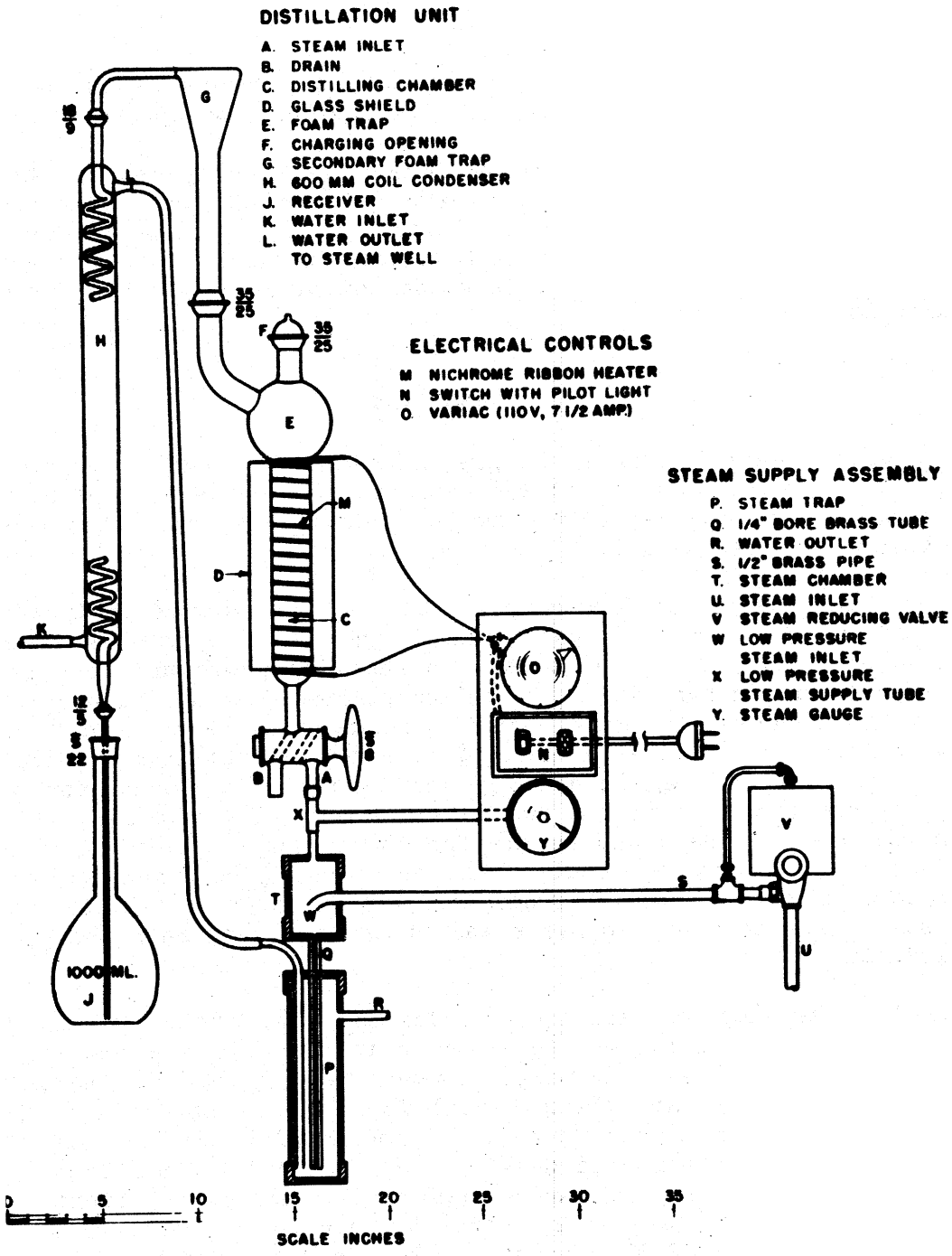
The procedure (3) for nicotine analysis, including both the distillation and the determination of nicotine, was modified, materially shortening the time required for analysis without sacrificing either accuracy or precision.

Before 1947, there were no data to indicate whether any change in the nicotine content occurs during drying. In 1946, therefore, nicotine analyses were made directly on the green leaves. The sample of green leaf blades (about 800 grams) was macerated and extracted in a Waring blender. The leaves were kept covered with acidified water (pH 1) to prevent possible loss of nicotine during the maceration. A weighed aliquot equivalent to 10 grams of dried leaf blade, as determined by moisture analysis, was transferred to an 800-ml. Kjeldahl flask. The nicotine was determined according to the procedure of the AOAC (3). The percentage nicotine was calculated on the moisture-free basis of the original green leaf blades.

The 1946 drying studies showed that *N. rustica* could be dried to a 12-15 percent residual moisture content without appreciable loss of nicotine. Therefore, a modified procedure for nicotine analyses of the green leaf blades was used in 1947 and 1948. Instead of analyzing the fresh leaves directly, they were first partly dried. About 1000 grams of green leaf blade material was dried at 65° C. to 12-15 percent moisture in a mechanical convection oven. At this moisture level, the leaves became friable and could be ground easily to pass a 20-mesh screen. The partly dried sample was then subdivided into "for analysis" samples, and the nicotine determined by the method described previously.

Nicotine determinations in the drying studies of 1946, 1947, and 1948 were made on samples ground to 20 mesh or finer. Two 10-gram portions were transferred to Kjeldahl flasks, and the nicotine contents were determined as described above.

Toward the latter part of the 1948 season, new methods of nicotine distillation and analysis were employed. The new distillation procedure used a Willits and Connelly modification (Fig. 1) of the apparatus of Griffith and Jeffrey (4). The modified still makes possible easier manipulation and cleaning, more complete condensation of distillate, and less breakage, and eliminates metallic contamination. Use of spherical joints facilitates cleaning and charging the apparatus and also lessens breakage, since they make the apparatus more flexible. A single distilling chamber together with the foam trap E replace the two chambers of the Griffith and Jeffrey still, resulting in more simple construction and reduced heating surfaces. An air shield prevents build up of latent heat from the heating coils wound on the walls of the distilling chamber and thus avoids the cracking of the chamber from the splashing of the solution, especially towards the end of the distillation. Use of the glass spiral condenser not only makes an all-glass apparatus but in addition provides sufficient cooling surface to completely condense all the distillate. The steam trap and steam chamber are made from copper pipe to minimize serious accidents that might result from escape of


DISTILLATION UNIT
A. STEAM INLET
B. DRAIN
C. DISTILLING CHAMBER
D. GLASS SHIELD
E. FOAM TRAP
F. CHARGING OPENING
G. SECONDARY FOAM TRAP
H. 600 MM COIL CONDENSER
J. RECEIVER
K. WATER INLET
L. WATER OUTLET TO STEAM WELL
ELECTRICAL CONTROLS
M NICHROME RIBBON HEATER
N SWITCH WITH PILOT LIGHT
O VARIAC (110V, 7 1/2 AMP)
STEAM SUPPLY ASSEMBLY
P. STEAM TRAP
Q. 1/4" BORE BRASS TUBE
R. WATER OUTLET
S. 1/2" BRASS PIPE
T. STEAM CHAMBER
U. STEAM INLET
V STEAM REDUCING VALVE
W LOW PRESSURE STEAM INLET
X LOW PRESSURE STEAM SUPPLY TUBE
Y. STEAM GAUGE
1000 ML.
SCALE INCHES

live steam. With this apparatus, distillations are completed in 15 minutes instead of the usual 2 to 3 hours required by the AOAC apparatus.

The nicotine in the distillate is determined by the spectrophotometric method of Willits et al. (5). This method, based on the measurement of the ultraviolet absorption maximum of nicotine at 259 mμ, reduces the time for the nicotine analysis to less than 15 minutes without loss of accuracy. The time for a complete analysis is less than 45 minutes, whereas formerly 2 to 3 days were required.

Sampling

Fresh Green Leaves. Since the green leaves were not the same in size, shape, or nicotine content, it was necessary to devise a sampling technique that would give representative samples identical in nicotine and moisture contents with those used in the drying studies.

In 1946, to obtain a uniform sample, the leaf blades were stripped from the midribs, cut into small 2-inch squares, and mixed. Since the freshly cut leaf squares lost moisture rapidly, a sampling procedure was needed that would permit rapid handling. The following procedure was adopted. Windrows of the cut squares were made, and 5-inch segments were taken from them at regular intervals until a sample of 2 or 3 kg. was obtained. These segments were composited and again quickly subdivided into three "for analysis" samples--one for moisture and two for nicotine. The remainder of the windrows was used as the gross sample for the drying studies. In the drying studies of 1946, erratic results were occasionally obtained, which could be explained only on the basis of slight differences in the actual moisture of the sample for moisture analysis and of the starting material used in the drying studies.

In 1947, a new sampling technique was designed and developed. The fresh, 2-inch sections of leaf blades, totaling about 2 kg., were placed in a 30-gallon air-tight drum and thoroughly mixed by rolling and tumbling. The sample for analysis and the material for the drying studies were taken immediately and simultaneously by the "grab-handful" method, transferred to covered tared containers and weighed. This technique resulted in samples for analysis and drying that were identical in moisture content. This was extremely important, because a 1 percent error in the moisture of the green material containing 82-89 percent moisture would cause an error of 5.5 to 9 percent when the nicotine content was calculated to a moisture-free basis.

Dried Leaf Blades. Sampling the dried leaf blades presented no particular problem. The friable blades were easily reduced to 20-mesh particles by grinding, without appreciable change of moisture content. The ground material was subsampled by successive passes through a Boerner sampler. The same procedure for sampling the dried products was followed in all subsequent studies.

Moisture Analysis

The moisture of the green leaf blade was determined at two temperatures. The green sample was partly dried at 65° C. in a mechanical convection oven, usually requiring 18 to 36 hours. Drying at this lower temperature tended to prevent case hardening of the leaf blades, and provide an embrittled sample that could be easily ground to facilitate mixing. Drying was completed at 110° in a forced-draft Brabender Rapid Moisture Tester. From the combined loss in weight that occurred at 65° and 110° C., the moisture content of the original green leaf blade was calculated by the following equation.

$$\% H_2O = [(100 - \% H_2O \text{ at } 65^\circ \text{ C.}) \times \frac{(\% H_2O \text{ at } 110^\circ \text{ C.})}{100}] + \% H_2O \text{ at } 65^\circ \text{ C.}]$$

Analyses of Air-Cured Tobacco and of Liquid Samples

The procedure for analyzing the air-cured tobacco samples was the same as that used for the dried products of the oven-drying studies. The liquid samples were distilled, and nicotine was determined as described in the nicotine determination section.

Statistical Analysis of the Nicotine Data of 1946 and 1947

A statistical comparison was made of the analytical data obtained in 1946 with those obtained in 1947 to find out whether the change in sampling technique resulted in improvement in precision. The precision was calculated from the differences between nicotine values of duplicates of the samples analyzed in 1946 and in 1947. Because the nicotine contents of the samples ranged from 1.3 to 10.2 percent on the moisture-free basis, it was necessary to place all these differences on a comparable 10 percent nicotine basis.

The 32 sets of duplicate nicotine values of the fresh leaf blades obtained in 1946 showed an average difference of 4.25 percent of the total nicotine present, with a standard error of ± 1.02 percent. The 16 sets of duplicate values obtained in 1947, when the partly dried leaf blade sampling technique was used, gave an average difference of only 0.85 percent, with a standard error of ± 0.60 percent. Since the same method for nicotine analysis was used in 1946 and 1947, any change in precision of the method was due to the change in sampling technique. To evaluate any change in precision gained by the new sampling technique used in 1947, an F test comparison was made of the differences between duplicate nicotine analyses for both seasons. In this test, developed by Youden (6), the mean value of squared differences, d, between duplicates of the pairs of nicotine values of the fresh leaf blades of 1946 was calculated by the following term (1), in which N_1 was the

32 pairs of duplicates.

(1) $$\frac{\Sigma d^2}{N_1}$$

A similar procedure was followed in calculating the differences, D, between the nicotine values of the fresh leaf blades in 1947. Here term (2) was used in which N_2 was the 16 pairs of duplicates of that group:

(2) $$\frac{\Sigma D^2}{N_2}$$

The ratio of (1) to (2) gave the F value (equation 3):

(3) $$F = \frac{\frac{\Sigma d^2}{N_1}}{\frac{\Sigma D^2}{N_2}}$$

The statistical significance of the calculated F was determined from the published tables of F values (7). The F test comparison for the two seasons shows that F = 25.74, a highly significant result, when it is considered that at the 1 percent level an F value of 3.20 is significant. This means that in less than 1 case out of 100 was the improvement in precision due to chance alone. Therefore, the method of analysis of the green leaf blades in 1947 was markedly superior in precision to that used in 1946.

A statistical comparison of nicotine values was also made to determine whether the new method of sampling the green material was as precise as that used for sampling the products of the drying studies. The values of the 45 sets of duplicates found for the dried products in 1947 had an average difference of 0.88 percent, with a probable error of ± 0.23 percent. An F test comparison between the differences of nicotine values of the dried products and the differences of the values of the green leaf blades for the same year gave an F value of only 1.28, which was not significant. Therefore, the nicotine analysis of the green leaf blades in 1947 had a precision equal to that of the dried leaf products indicating that the method of sampling the green leaf blades was as precise as the method of sampling the dried leaf blade products.

A graphic summary of the nicotine analyses showing differences between duplicates of the different kinds of samples and the different seasons is shown in figure 2. Since the differences between duplicates for 1947 were clustered close to zero, little was to be gained by attempted improvement of the methods of analysis, and therefore the method used in 1947 was used in 1948.

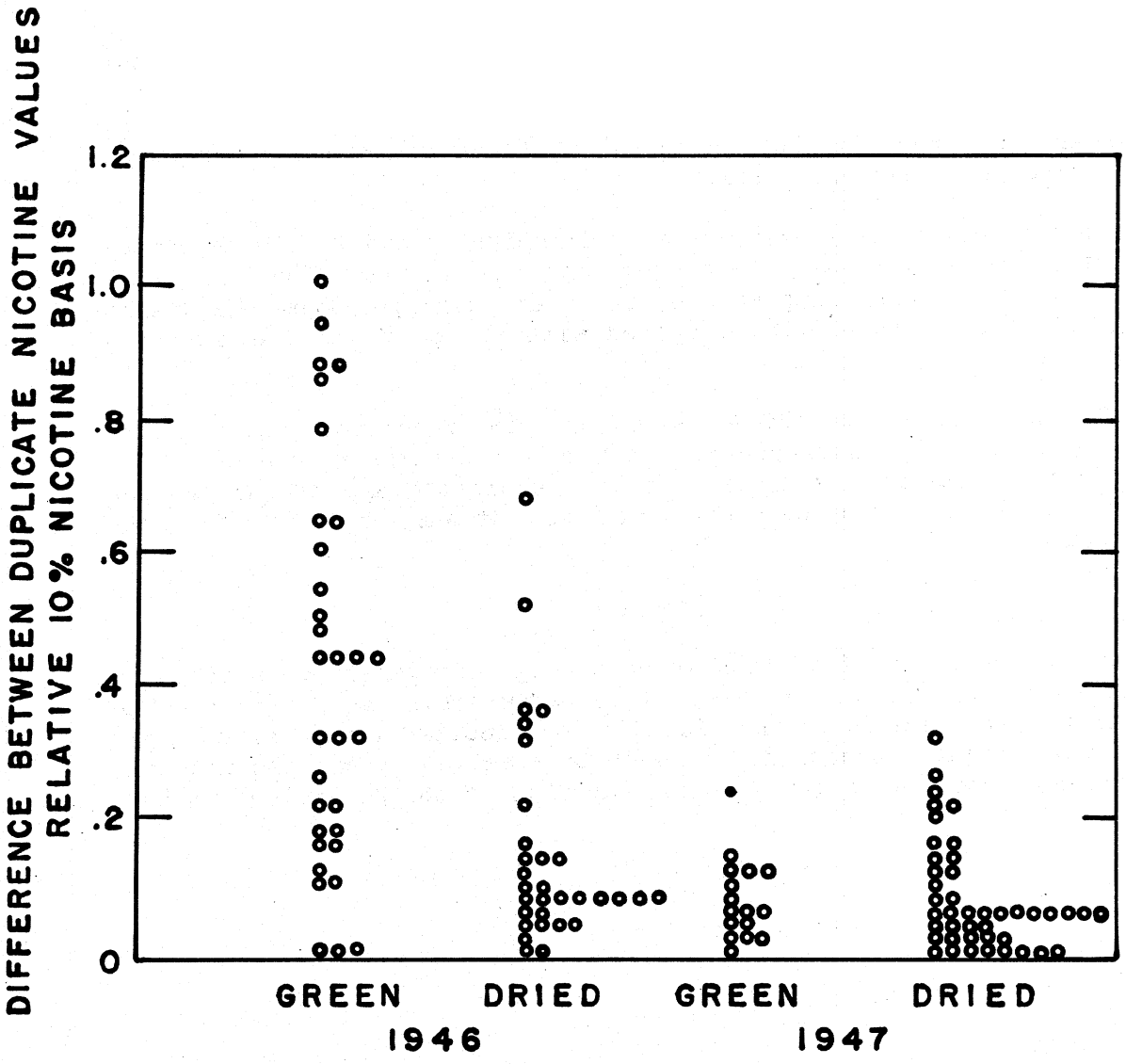


Figure 2.--Graphic summary of nicotine analysis showing effect of sampling procedures used in 1946 and 1947.

AIR DRYING

A series of experiments was made to determine to what extent the nicotine content is affected by slowly air drying *N. rustica*. Air drying could easily be practiced by a farmer equipped with an ordinary tobacco shed.

Some of the *N. rustica* grown in each of the 3 years was harvested, and the whole plants were placed on sticks and hung in a barn. No artificial heat was supplied. The leaves, including the midribs or stems, cured in 4 to 6 weeks; the stalks required 13 to 17 weeks. The dried product had a good appearance and was free of mold, which is sometimes observed in air-cured tobaccos during moist curing seasons.

Great care was taken to select samples that in maturity and size were comparable with the plants taken for the oven-drying experiments. The nicotine contents of the barn-dried samples when they were stripped from the stalks 4 or 5 months after harvest were compared with those of the freshly harvested samples.

Variety 34753 was used in the 1946 experiments. The cured material analyzed was the leaf blade only, stripped of the midribs; it was comparable with that used in the oven-dried samples. Table 1 shows the age of the samples when harvested, and the nicotine contents at harvest and after 4 to 5 months of barn drying.

(Table 1)

Considering the obvious difficulties in obtaining representative samples it can be concluded that there is no loss of nicotine during barn drying. The data also show that there is no translocation of nicotine from leaf to stalk during the slow drying process, for if such movement occurred, the nicotine figures for the cured leaf would be less than that of the green leaf at harvest.

Table 1. Nicotine contents of green and barn-cured rustica, variety 34753, and yields of nicotine per acre. Leaf blades only, crop of 1946.

Days in Field 1/	Nicotine content			
	Nicotine per acre	Green	Barn Cured	Loss or gain
	Lbs.	%2/	%2/	%
74	89		7.71	
77	74	7.58	6.78	-0.80
82	90	7.11	7.19	.08
88	130	8.66	8.65	- .01
92	138	8.48	8.93	.45
97	152	9.11	9.02	- .09
Average				- .07

1/ From date seedlings were transplanted to field (May 24) to date sample was harvested.

2/ Moisture-free basis.

In 1947, the variety Olson 68 was used. The data are given in table 2.

Table 2. Nicotine contents of green and barn-cured rustica, variety Olson 68, and yields of nicotine per acre. Leaf blades and midribs, crop of 1947.

Days in Field[1]	Harvested leaves per acre	Nicotine content			
		Nicotine per acre	Green	Barn-cured	Loss or Gain
	Lbs.	Lbs.	%[2]	%[2]	%
		First setting, June 6			
70	2100	162	6.60	7.74	1.14
80	2470	176	7.40	7.16	- .24
88	1980	148	7.28	7.49	.21
98	2257	177	7.52	7.88	.36
Average					.37
		Second setting, June 17			
90	1970	150	7.61	7.64	.03
97	2148	154	8.08	7.18	- .90
108	2473	164	6.96	6.66	- .30
Average					- .39

1/ From date seedlings were planted to date sample was harvested.

2/ Moisture-free basis.

Inadvertently, the green samples were leaf blades only, whereas the barn-cured samples included the midribs. Since the midribs constitute about one-fifth of the whole leaf and contain about one-half as much nicotine, this could explain the lower average nicotine content of the barn-dried material of the June 17 planting (table 2). The June 6 planting showed a higher nicotine average in the barn-dried material. These inconsistencies can best be explained by sampling error. It is concluded that there is no evidence here for loss of nicotine during barn drying.

In 1948, the Olson 68 variety was used again, but comparisons were made of leaf blades only. The data are given in table 3. Again there was no difference in nicotine content between the fresh and dried samples.

(Table 3)

Table 3. Nicotine contents of green and barn-cured rustica, variety Olson 68, and yields of nicotine per acre. Leaf blades and midribs, crop of 1948.

Days in Field[1/]	Nicotine content			
	Nicotine per acre	Green	Barn-cured	Loss or gain
	Lbs.	%[2/]	%[2/]	%
82	119	5.19	6.05	0.86
88	132	6.31	6.87	.56
91	140	7.57	6.32	-1.25
95	215	7.54	7.54	0
98	155	7.35	6.90	- .45
101	211	7.70	8.14	.44
Average				.03

1/ From date seedlings were transplanted to field (June 10) to date sample was harvested.

2/ Moisture-free basis.

We conclude that in the Philadelphia area *N. rustica* leaves can be air-cured without use of heat. The leaf blades require 4 to 6 weeks; the stalks require 4 months. It is thus similar to our common air-cured tobaccos. During the slow cure, there is no translocation of nicotine from the leaf to the stalk, nor is there any loss directly from the leaf.

OVEN DRYING

C. F. Woodward and C. O. Badgett

In the oven-drying studies, the oven was adjusted so that heated air was driven upward through a bed of leaf tissue on a screen-bottomed tray, 18 by 36 inches, before expulsion from the drying chamber. A motor-driven fan circulated the heated air at a linear velocity of 1100 feet per minute.

Only leaf blades were used. The freshly harvested leaf tissue was cut into approximately 2-inch squares to facilitate sampling and drying. The sampling and analytical procedures employed in this study were the same as those described earlier in the paper.

The laboratory drying experiments were carried out during three successive growing seasons, and experience gained from one year's results was applied to modify and refine the experiments in the following season. Because of these progressive changes in objectives and experimental techniques, the procedures will be discussed chronologically.

Drying Experiments in 1946. To minimize errors inherent in the sampling, analytical, and drying procedures, over-all averages of nicotine losses and residual moisture contents were obtained in triplicated experiments involving three drying conditions. These conditions, arbitrarily chosen after preliminary trials, were 55 minutes at about 90°-140°C.; 6.75 hours at 45°-55°; and 24 hours at 25°-35°. In the first two sets of experiments, the leaves were removed from the oven and "tossed" after the first and second third of the drying period. Tossing of the leaves was omitted in the 24-hour experiments.

Table 4 shows the data obtained in the drying experiments in 1946.

Table 4. Data obtained in drying experiments on *N. rustica* leaf blades in 1946

Days in field	Drying conditions °C.	Hours	Moisture Green %	Moisture Dried %	Nicotine Green %[1]	Nicotine Dried %[1]	Nicotine Loss %
			Variety 34753; transplanted May 22				
77	42- 56	6.7	89.8	12.5	7.58	7.31	3.1
82	110-148	0.9	89.3	2.5	6.25	5.61	10.2
82	25- 35	26.0	88.5	7.4	7.97	8.22	-3.1
88	88-130	0.9	90.1	7.5	8.61	8.02	6.8
88	25- 36	24.0	88.9	11.7	8.66	8.66	0
90	44- 55	6.7	89.3	7.7	8.48	8.06	5.0
96	100-138	0.9	87.9	7.0	9.14	8.22	10.1
96	45- 55	6.7	88.1	7.7	9.76	9.18	6.3
97	25- 35	24.0	87.6	13.4	9.11	8.95	1.8
			Variety 23; transplanted June 18				
63	95-135	0.9	87.0	2.2	3.46	3.57	0.8
65	26- 38	24.0	88.0	9.2	3.75	3.82	-2.8
69	42- 56	6.7	88.1	8.0	4.61	4.41	4.3
83	80-130	0.9	86.2	4.8	6.23	5.78	7.2
84	44- 54	6.7	87.6	16.4	7.07	6.93	2.0
85	25- 35	24.0	85.3	13.4	6.88	6.69	2.8
97	90-138	0.9	87.0	2.9	9.85	8.86	10.0
98	42- 55	6.7	88.4	16.4	9.24	9.14	1.1
99	25- 35	24.0	88.7	12.2	9.96	9.63	3.3
			Variety 34753; transplanted July 24				
68	95-137	0.9	86.8	1.6	3.33	3.25	2.4
69	45- 55	6.7	85.8	4.6	4.49	4.39	2.2
70	25- 35	24.0	85.0	8.6	3.93	4.39	-10.5
81	25- 35	24.0	84.5	6.5	5.04	5.19	-3.0
82	95-140	0.9	84.4	0.5	5.95	4.97	16.6
84	45- 56	6.7	86.3	5.0	6.44	5.95	7.6
95	25- 35	24.0	88.3	10.6	6.04	5.96	1.3
96	88-140	0.9	85.9	3.7	6.33	5.63	11.6
97	45- 55	6.7	85.4	6.2	6.24	6.20	0.6

1/ Moisture-free basis.

The percentage of nicotine loss on drying was calculated by the equation

$$\% \text{ nicotine lost} = 100 \frac{(\%\text{nicotine, MFB, green} - \%\text{ nicotine, MFB, dried})}{\%\text{ nicotine, MFB, green}}$$

Over-all averages of nicotine losses and residual moisture contents, recorded in table 5, give equal weight to each of the individual drying experiments without consideration of variety, age at harvest, and the moisture and nicotine contents of the green plants. The over-all average nicotine loss after 24 hours' drying at 25-35° C. was -1.0 percent, or an apparent 1.0 percent gain in the total nicotine of the dried product over the green material (table 5). Since this was probably due to experimental errors, it is concluded that the loss incurred under these drying conditions was negligible. When the leaf blades were dried at 45-55° for 6.75 hours, the average loss of nicotine was 3.6 percent, whereas at 90-140° for 55 minutes it was 8.0 percent. Thus, the loss seemed to increase at the higher temperatures.

(Table 5)

At the same time, table 5 shows that the loss of nicotine was inversely proportional to the residual moisture content, for with moisture contents of 10.3, 9.4, and 3.6 percent there was a loss of nicotine of -1.0, 3.6, and 8.0 percent, respectively. If the data are divided into two groups on the basis of residual moisture content, disregarding the drying conditions, another comparison can be made. The average residual moisture content for all experiments was 7.79 percent. The 11 experiments in which the moisture content was above this value, with an average of 12.05 percent, show an average nicotine loss of 1.0 percent. The 16 experiments having a residual moisture content below 7.79 percent, with an average of 4.8 percent, show an average nicotine loss of 5.5 percent.

On the basis of the data obtained in 1946, with an average of 4.8 percent, it was tentatively concluded that the loss of nicotine from *Nicotiana rustica* leaf tissue on drying is proportional to the moisture removed.

Drying Experiments in 1947. To demonstrate more conclusively the relationship between loss of nicotine and extent of drying, attempts were made to dry equivalent green samples to residual moisture contents of 3, 6, and 12 percent. The 12 percent moisture content was chosen as the upper limit because it approximates the maximum for storage without spoilage.

In each experiment, 2000 grams of green leaf was used, an amount producing a leaf mat about 3 inches deep on the drying tray.

Three sets of drying experiments, designed to produce products of about 3, 6 and 12 percent moisture, were conducted on each green sample. In the first, the filled tray was placed in the heated oven, and the time was recorded. After 10 minutes, the tray was reversed from left to right. After 20 minutes, the tray was removed, and the partly dried leaves were tossed

Table 5. Average nicotine losses and residual moisture contents of *N. rustica* dried under three conditions in 1946.

	Set	24 hrs. at 25°-35°C.		6.75 hrs. at 45°-55°C.		0.92 hr. at 90°-104°C.	
		Nicotine loss	Moisture	Nicotine loss	Moisture	Nicotine loss	Moisture
		%	%	%	%	%	%
				Planting 1			
	1	- 3.1	7.4	3.1	12.5	10.2	2.5
	2	0	11.7	5.0	7.7	6.8	7.5
	3	1.8	13.4	6.3	7.7	10.1	7.0
	Average	- 0.4	10.8	4.8	9.3	9.0	5.6
				Planting 2			
	1	- 1.9	9.2	4.3	8.0	- 3.2	2.2
	2	2.8	13.4	2.0	16.4	7.2	4.8
	3	3.3	12.2	1.1	16.4	10.0	2.9
	Average	1.4	11.6	2.5	13.6	4.7	3.3
				Planting 3			
	1	-10.5	8.6	8.2	4.6	2.4	1.6
	2	- 3.0	6.5	7.6	5.0	16.6	0.5
	3	1.3	10.6	0.6	6.2	11.6	3.7
	Average	- 4.1	8.6	3.5	5.3	10.2	1.9
Over-all	Average	- 1.0	10.3	3.6	9.4	8.0	3.6

and redistributed on the tray. After 30 minutes, the tray was again reversed. After 40 minutes, the tossing procedure was repeated. The charge was then dried for an additional 50 minutes, or a total drying time of 90 minutes. The tray was weighed after each tossing, and at the end of the experiment. The oven temperature was thermostatically controlled so that the maximum temperature of the air was 120° C. (248° F.). On the basis of preliminary experiments, it was assumed that the material dried in this manner contained 3 percent moisture, and the weights corresponding to 6 and 12 percent moisture were calculated. Drying procedures for the second and third sets, aimed at 6 and 12 percent moisture, were identical with that employed for the 3 percent moisture sample for the first 40 minutes. After 40 minutes, the trays were removed periodically and weighed until the calculated weight was attained. Thirteen series, involving two plantings, were processed by this procedure.

Table 6 gives the data for 1947. In table 7, they are regrouped to show the relation between the residual moisture contents and the corresponding nicotine losses for 13 series, each comprising 3 experiments.

(Tables 6 and 7)

As anticipated from the previous year's results, the samples dried to the lowest moisture content showed the greatest loss of nicotine. The average residual moisture contents and losses of nicotine were 2.7 and 6.7; 6.7 and 3.6; 11.0 and 4.7, respectively.

As another means of showing possible relations between temperature, final moisture, and loss of nicotine, the coefficient of correlation by rank was calculated by the following formulas.

$r = 1 - \frac{6\Sigma D^2}{n(n^2-1)}$; $e = 0.706. \frac{1 - r^2}{\sqrt{n}}$, where n is the number of experiments, D is the difference in rank, and e is the probably error.

When loss of nicotine and residual moisture are compared, the value for r equals 0.128 ± 0.111. Since r should be about three times the probable error to be significant, we must conclude that final moisture *per se* is not the controlling factor in loss of nicotine.

When maximum drying temperature and nicotine loss in the 39 drying experiments were compared, the value for r was 0.317 ± 0.101, indicating that the maximum drying temperature is a critical factor in loss of nicotine.

Furthermore, in the 19 experiments in which the maximum temperature of the air was above 108° C., the average loss of nicotine was 6.3 percent. In the 20 experiments in which the maximum temperature was 108° or lower, the average loss of nicotine was 3.6 percent.

Table 6. Data obtained in drying experiments on *N. rustica* leaf blades in 1947.

Date of Drying	Days in field	Drying conditions °C.	Drying conditions Minutes	Weight of dried Material Grams	Moisture green %	Moisture dried %	Nicotine green %[1]	Nicotine dried %[1]	Nicotine Loss %
				Transplanted June 6					
8-15	70	91-116	90	253	87.8	3.0	6.60	6.39	3.2
		96-114	40	284	"	13.2	"	6.24	5.4
		94-116	48	262	"	6.4	"	6.49	1.7
8-25	80	94-119	90	244	88	3.8	7.4	6.58	11.1
		97-112	51	262	"	11.5	"	6.70	9.5
		94-109	50	245	"	5.4	"	6.88	7.0
8-29	84	86-120	90	219	89.2	2.1	6.73	6.34	5.8
		90-100	40	241	"	9.8	"	6.76	-0.4
		88-101	50	227	"	5.7	"	6.76	-0.4
9-2	88	90-120	90	246	88.2	2.6	7.28	6.62	9.1
		91-106	44	273	"	11.5	"	6.77	7.0
		89-103	45	262	"	7.6	"	6.59	9.5
9-8	94	83-119	90	248	88.0	2.8	8.13	7.43	8.6
		92-109	40	280	"	13.3	"	7.87	3.2
		92-108	45	266	"	9.0	"	7.80	4.1
9-12	98	93-120	90	254	87.4	3.2	7.52	7.20	4.[illegible]
		96-106	41	283	"	11.8	"	7.51	0.[illegible]
		91-106	49	262	"	6.1	"	7.56	-0.5
9-16	104	87-118	90	264	86.9	2.0	7.78	7.38	5.1
		94-107	40	291	"	10.4	"	7.79	-0.1
		83-105	60	270	"	4.4	"	7.72	0.8
9-23	109	89-120	90	297	85	1.8	7.10	6.81	4.1
		84-102	43	328	"	9.6	"	6.70	5.6
		85-102	50	309	"	5.9	"	7.08	0.3
9-30	116	89-120	90	307	85.0	1.7	6.01	5.95	1.0
		93-104	39	333	"	8.8	"	5.88	2.2
		83-100	49	321	"	5.8	"	6.20	-3.2
				Transplanted June 17					
9-10	85	84-119	90	237	88.5	3.0	7.47	6.89	7.8
		90-110	49	259	"	11.[illegible]	"	7.04	5.8
		85-101	50	249	"	8.0	"	7.25	2.9
9-17	92	89-119	90	257	87.5	3.0	7.61	7.00	8.0
		89-102	41	288	"	12.4	"	7.01	7.9
		86-98	53	265	"	7.8	"	7.08	7.0
9-22	97	80-116	90	255	87.7	3.8	8.08	7.15	11.5
		86-101	50	280	"	11.8	"	7.40	8.4
		91-108	48	270	"	9.6	"	7.21	10.8
9-29	104	89-122	90	315	84.0	2.5	7.57	6.96	8.1
		89-106	43	345	"	8.8	"	7.07	6.6
		88-105	51	329	"	6.1	"	7.05	6.9
10-1	106	85-100	150	370	82.0	2.6	6.96	6.82	2.0
		"	50	387	"	6.4	"	6.71	3.6
		"	400	373	"	2.8	"	6.77	2.7

Table 7. Nicotine losses and residual moisture contents of *N. rustica*, variety 68, dried in 1947. (Data of table 6)

Approximately 3 percent residual moisture		Approximately 6 percent residual moisture		Approximately 12 percent residual moisture	
Residual moisture	Nicotine loss	Residual moisture	Nicotine loss	Residual moisture	Nicotine loss
%	%	%	%	%	%
		Transplanted June 6			
3.0	3.2	6.4	1.7	13.2	5.4
3.8	11.1	5.4	7.0	11.5	9.5
2.1	5.8	5.7	-0.4	9.8	-0.4
2.6	9.1	7.6	9.5	11.5	7.0
2.8	8.6	9.0	4.1	13.3	3.2
3.2	4.2	6.1	-0.5	11.8	0.1
2.0	5.1	4.4	0.8	10.4	-0.1
1.8	4.1	5.9	0.3	9.55	5.6
1.7	1.0	5.8	-3.2	8.8	2.2
		Transplanted June 17			
2.7	7.8	7.7	2.9	10.7	5.8
2.9	8.0	7.8	7.0	12.4	7.9
3.8	11.5	9.6	10.8	11.8	8.4
2.5	8.1	6.1	6.9	8.8	6.6
Av. 2.7	6.7	6.7	3.6	11.0	4.7

As a further test of correlation between maximum drying temperature and loss of nicotine, the experiments in which the final moisture objectives were 6 or 12 percent were divided into two groups of 13 each, in which the respective maximum temperature ranges were 98° to 105° and 106°to 116° C. Average losses of nicotine were 3.7 percent for the former and 4.7 percent for the latter.

Although it was originally intended to maintain a constant oven temperature of 110°-120° C., the maximum temperature of 120° was obtained only in the samples dried to about 3 percent moisture. Presumably, the heating capacity of the oven was sufficient for maintenance of an air temperature of 120° only after the material had been dried to about 6 percent moisture. These considerations suggested the final series of experiments--on October 1--in which the maximum drying temperature was 100° and the samples were dried for 50, 150, and 400 minutes. The fact that with residual moisture contents of 6.4, 2.6, and 2.8 percent losses of nicotine were only 2.0, 3.6, and 2.7 percent, respectively, indicates that drying temperature is an important factor in loss of nicotine. Although it is realized that the air temperature is not as reliable a gauge as the leaf temperature, toward the end of the drying periods at the above moisture contents, the two were probably the same.

Drying Experiments in 1948. Since there seemed to be a significant relation between maximum drying temperature and loss of nicotine in the drying experiments in 1947, it was deemed advisable in 1948 to employ experimental conditions that would more clearly demonstrate this relation. Equivalent samples of leaf tissue were dried to approximately 3 percent residual moisture at 75-90°, 95-110°, and 115-130° C. To approximate commercial drying practice, a fourth sample in each series was dried to about 12 percent residual moisture at 105-120°. The drying procedures were similar to those employed in the 1947 experiments in that the samples were "tossed" and redistributed on the tray at least once during the drying process. In the experiments at 75-90°, the samples were tossed after 30 minutes and 60 minutes; at 95-110°, after 20 minutes; at 115-130° and at 105-120°, after 10 minutes.

Table 8 gives the complete drying data. The residual moisture contents and the corresponding nicotine losses for the four experiments in each of the seven series are shown in table 9. As in the previous 2 years' experiments, appreciable differences in loss of nicotine were obtained under nearly identical drying conditions. Because of this variation between individual experiments, consideration is given only to the averages of the seven replicates. These average values--0.1 percent at 75-90° C., 1.3 percent at 95-110°, and 2.5 percent at 115-130°--for the samples of approximately 3 percent residual moisture suggest that nicotine losses are directly proportional to the drying temperature, although the average nicotine losses for the higher drying temperatures were lower than anticipated.

Table 8. Data obtained in drying experiments on *N. rustica* leaf blades in 1948

Date of Drying	Days in field	Drying conditions °C.	Drying conditions Min.	Moisture Green %	Moisture Dried %	Nicotine Green %1/	Nicotine Dried %1/	Nicotine Loss %
9-1	82	(75- 90	90	86.8	3.7	5.19	5.75	-10.8
		(92-110	40	"	3.2	"	5.75	-10.8
		(115-130	18	"	3.6	"	5.53	- 6.6
		(105-120	19	"	14.4	"	5.61	- 8.1
9-7	88	(75- 91	100	87.5	4.0	6.31	6.36	- 0.8
		(95-110	42	"	3.2	"	6.30	0.16
		(115-130	18	"	4.9	"	6.30	0.16
		(105-120	21	"	6.1	"	6.02	4.6
9-10	91	(75-91	100	86.3	4.1	7.57	7.42	2.0
		(95-100	42	"	3.4	"	7.11	6.1
		(115-130	18	"	4.0	"	6.84	9.6
		(105-120	20	"	7.1	"	7.24	4.4
9-14	95	(75- 90	105	86.6	3.3	7.54	7.18	4.8
		(95-110	42	"	2.9	"	7.30	3.2
		(115-130	18	"	7.1	"	7.19	4.6
		(100-120	18	"	9.0	"	7.36	2.4
9-17	98	(75- 90	110	86.2	3.7	7.35	7.40	- 0.7
		(90-110	42	"	3.5	"	7.44	- 1.2
		(115-130	24	"	4.0	"	7.24	1.5
		(105-120	16	"	12.8	"	7.02	4.5
9-20	101	(74- 90	110	85.9	4.5	7.70	7.56	1.8
		(91-110	42	"	4.3	"	7.35	4.5
		(115-130	26	"	4.8	"	7.39	4.0
		(105-120	21	"	10.9	"	7.13	7.4
9-24	105	(75- 90	110	84.6	3.7	8.59	8.18	4.8
		(95-110	42	"	3.3	"	8.00	6.9
		(113-130	23	"	3.6	"	8.23	4.2
		(105-120	18	"	12.8	"	8.23	4.2

Table 9. Drying temperatures, residual moisture contents, and nicotine losses of *N. rustica* in experiments in 1948. (Data of table 8)

Expt. No.	Approximately 3% residual moisture						Approximately 12% residual moisture	
	Dried at 75°-90°C.		Dried at 95°-110°C.		Dried at 115°-130°C.		Dried at 105°-120°C.	
	Residual moisture	Nicotine loss	Residual moisture	Nicotine loss	Residual moisture	Nicotine loss	Residual moisture	Nicotine loss
	%	%	%	%	%	%	%	%
67-70	3.7	-10.8	3.2	-10.8	3.6	-6.6	14.4	-8.1
71-74	4.0	- 0.8	3.2	0.2	4.9	0.2	6.1	4.6
75-78	4.1	2.0	3.4	6.1	4.0	9.6	7.1	4.4
79-82	3.3	4.8	2.9	3.2	7.1	4.6	9.0	2.4
83-86	3.7	- 0.7	3.5	- 1.2	4.0	1.5	12.8	4.5
87-90	4.5	1.8	4.3	4.5	4.8	4.0	10.9	7.4
91-94	3.7	4.8	3.3	6.9	3.6	4.2	12.8	4.2
Average	3.8	0.1	3.4	1.3	4.6	2.5	10.4	2.8

From a practical standpoint, the average nicotine losses in drying to approximately 10-13 percent residual moisture at temperatures of 105-120° C. (220-248° F.) are most deserving of consideration. In the 10 experiments made under these conditions in 1947 and 1948, the average nicotine loss was 5.5 percent. None of the 1946 samples was dried in this manner.

HOW IS NICOTINE LOST DURING THE DRYING PROCESS ?

C. F. Woodward

Nicotine can be lost during drying by volatilization, by chemical change, or by both. The drying oven used in the laboratory experiments could not be equipped with an efficient scrubber for the exhaust gases, and consequently it was impossible to obtain a nicotine and nitrogen balance on the green and dried material.

The problem was studied, however, in the following equipment. The sample was placed in a large glass U-tube (4.5 by 14 inches; internal diameter, 1.25 inches) immersed in an oil bath heated to 140° C. The inlet to the glass U-tube was connected to an orifice flowmeter, from which air was passed through the sample at the rate of 8 mol per hour. The outlet of the tube was attached to a water-cooled condenser, which discharged into a 1-liter two-necked flask. The flask was equipped with a vertical scrubber filled with glass beads. During the drying process, 5 percent hydrochloric acid was allowed to trickle down through the scrubber countercurrent to the upward passage of exhaust gases. All the nitrogenous components in the air stream were recovered in this manner.

The sample used in each of the six drying experiments listed in table 10 consisted of 80 grams of a previously dried batch of leaf meal of 10-mesh particle size and containing 7.3 percent moisture. The nitrogen and nicotine contents, on a moisture-free basis, were 4.98 and 7.36 percent, respectively. Data obtained in periodic check analyses during the series of experiments did not vary significantly from these values.

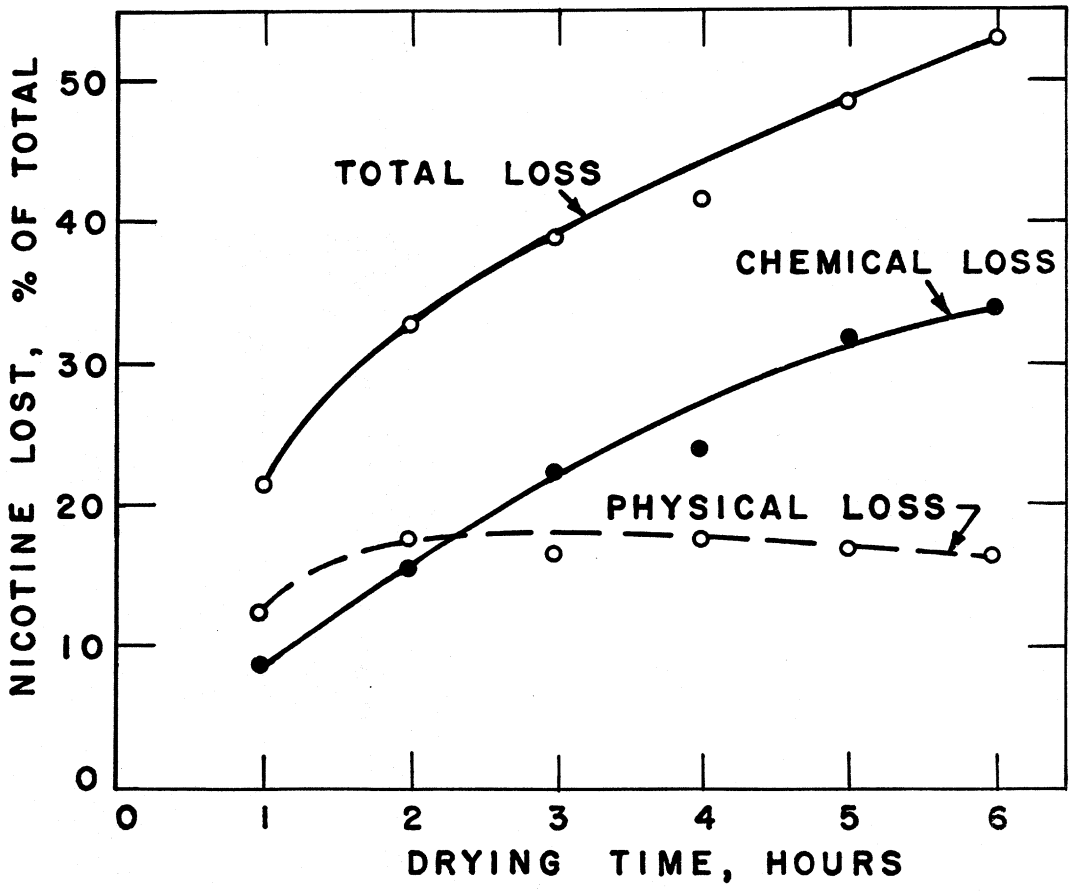
(Table 10)

After air had been passed through the charge for the indicated time, the U-tube was removed from the oil bath and allowed to cool to room temperature. The residual sample was then weighed and then analyzed for nicotine and nitrogen. The distillate and acid scrubbing solution were combined, and also analyzed for nicotine and nitrogen. These analyses provided the basis for the calculated weight and percentage values given in table 10.

As the time of heating was increased from 1 to 6 hours the nicotine lost from the charge increased progressively from 1.25 grams (21.4 percent) to 2.91 grams (50.3 percent). The nicotine recovered in the distillate and acid scrubber increased from 0.73 gram (12.5 percent) after 1 hour to 1.02 grams (17.5 percent) after 2 hours. This physical loss of nicotine from the charge did not increased after 2 hours of heating. A possible explanation

Table 10. Nitrogen and nicotine balance in drying 80 grams (M.F.B.) of *N. rustica* at 140° C.

Time of heating Hours	Nicotine				Nitrogen			
	In charge grams	Lost from charge % of total	In distillate and scrubber % of total	Unaccounted for % of total	In charge grams	In charge after heating grams	In distillate and scrubber (nicotine equivalent, N x 5.8) grams	Recovery %
1	5.84	21.4	12.5	8.9	3.98	3.89	0.13 (0.75)	101.0
2	5.84	32.7	17.5	15.2	3.98	3.85	0.19 (1.10)	101.5
3	5.86	38.9	16.7	22.2	3.99	3.83	0.18 (1.04)	100.5
4	5.78	41.5	17.5	24.0	3.99	3.81	0.18 (1.04)	100.0
5	5.78	48.8	17.0	31.8	3.99	3.80	0.17 (0.99)	99.5
6	5.78	50.3	16.5	33.8	3.99	3.76	0.16 (0.93)	98.2


NICOTINE LOST, % OF TOTAL
0
10
20
30
40
50
0
1
2
3
4
5
6
DRYING TIME, HOURS
TOTAL LOSS
CHEMICAL LOSS
PHYSICAL LOSS

for the cessation of nicotine volatilization after the 2-hour heating period is that the loss of basic nicotine results in a more acidic residue and complete fixation of the remaining nicotine.

The loss of nitrogen increased progressively with the increase in heating time from 1 to 6 hours. The nitrogen in the distillate and acid scrubber was comparable to the nicotine evolved in that the quantity was fairly constant for two or more hours of heating. Furthermore, a close quantitative correlation was found between the actual weight of nicotine determined by analysis and the weight calculated by multiplying the nitrogen value by the theoretical nicotine conversion factor of 5.8. In other words, nicotine accounted for essentially all the nitrogen lost from the charge.

The percentage of nitrogen accounted for ranged from 98.2 to 101.5 percent, and averaged 100.1 percent. This excellent nitrogen balance demonstrates that there was no loss of nitrogen or nitrogen-containing compounds from the system. Hence, the failure to obtain a nicotine balance can be explained only on the basis of a chemical change in some of the nicotine. The nicotine unaccounted for was designated as the "chemical loss," in contrast to the "physical loss," or nicotine volatilized and recovered in the receiving system. The total loss of nicotine was the difference in nicotine content of the original charge and residual material as determined by spectrophotometric analysis (5). Figure 3 shows the physical, chemical, and total losses of nicotine in these drying experiments.

Since the chemical loss of nicotine becomes appreciable only after prolonged heating at 140° C., it is assumed that losses of nicotine under the milder drying conditions employed in 1946, 1947, and 1948 were predominantly physical.

Whether or not the chemical loss of nicotine under these conditions results in the formation of products similar to those produced during the fermentation of cigar tobacco (8) can be decided only by further experiments.

DISTRIBUTION OF NICOTINE IN *NICOTIANA RUSTICA*

Constantine Ricciuti, C. O. Willits and E. G. Beinhart

Methods

Two mature plants of the Brazilian type (variety T. I. 23), typical as to normal plant growth and leaf characteristics, were used in these distribution studies. The plants were selected from a field in which the seedlings had been set out on June 18. One of the plants (A) was selected from a plot in which the plants had been topped and suckered on August 14. The secondary growth of suckers, which normally occur in the leaf axils, had been removed on August 30. In an adjoining plot, in which the plants had been left intact, another plant (B) was selected. Plants A and B were judged (on August 18) to be equal in size and leaf measurement. Both plants were 30 inches high up to the "first bald sucker," at which point plant A was topped. The plants were harvested when it was decided that the maximum


36


42

nicotine content had been attained. This was 84 days after planting for A and 79 days after planting for B (figures 4 and 5).

The plants were harvested with a ball of earth to prevent excessive loss of roots, and the adhering soil was removed by washing in a stream of water. The excess water was blotted off, and the plants were weighed as rapidly as possible.

The leaves of each plant were counted, and the stalks of the two plants were cut so that there was an equal number of leaves on the upper half and lower half sections. The roots were cut off at the ground line. The topped and suckered plant (A) was subdivided into the following plant sections: upper half leaves, lower half leaves, upper half stalk and lower half stalk, and the leaves were further subdivided into midribs and petioles and leaf blade or laminae. For the unpruned plant (B), besides these subdivisions there was a further subdivision into main plant seed heads, sucker stalks, sucker leaves, and sucker seed heads. The sucker leaves were also divided into midribs and petioles and leaf blades. The sections were sampled, and replicates were used for moisture and nicotine analyses.

Results

Table 11 gives the analysis of the topped and suckered plant A. The data show the weight in grams, the percentage weight, the percentage moisture, and the percentage nicotine on a moisture-free basis of these sections. Data are included on the percentage distribution of all the nicotine in the plant exclusive of that in the roots.

(Table 11)

The table shows that 63.4 percent of the total weight of the green plant was in the leaves, 7.6 percent in the roots, and 29.0 percent in the stalk. The percentage moisture had a narrow range--from 82.4 percent in the lower half stalk to 91.0 percent in the midribs and petioles of the lower half. Curiously enough, the stalk and leaves had almost the same moisture content.

The part of the plant having the greatest percentage of nicotine was the leaves section, which contained 8.48 percent nicotine; the leaf blades of the upper half contained 10.76 percent nicotine. The petioles and midribs had relatively less nicotine, 2.10 in the lower half and 2.41 percent in the upper half. The stalk contained 2.13 percent.

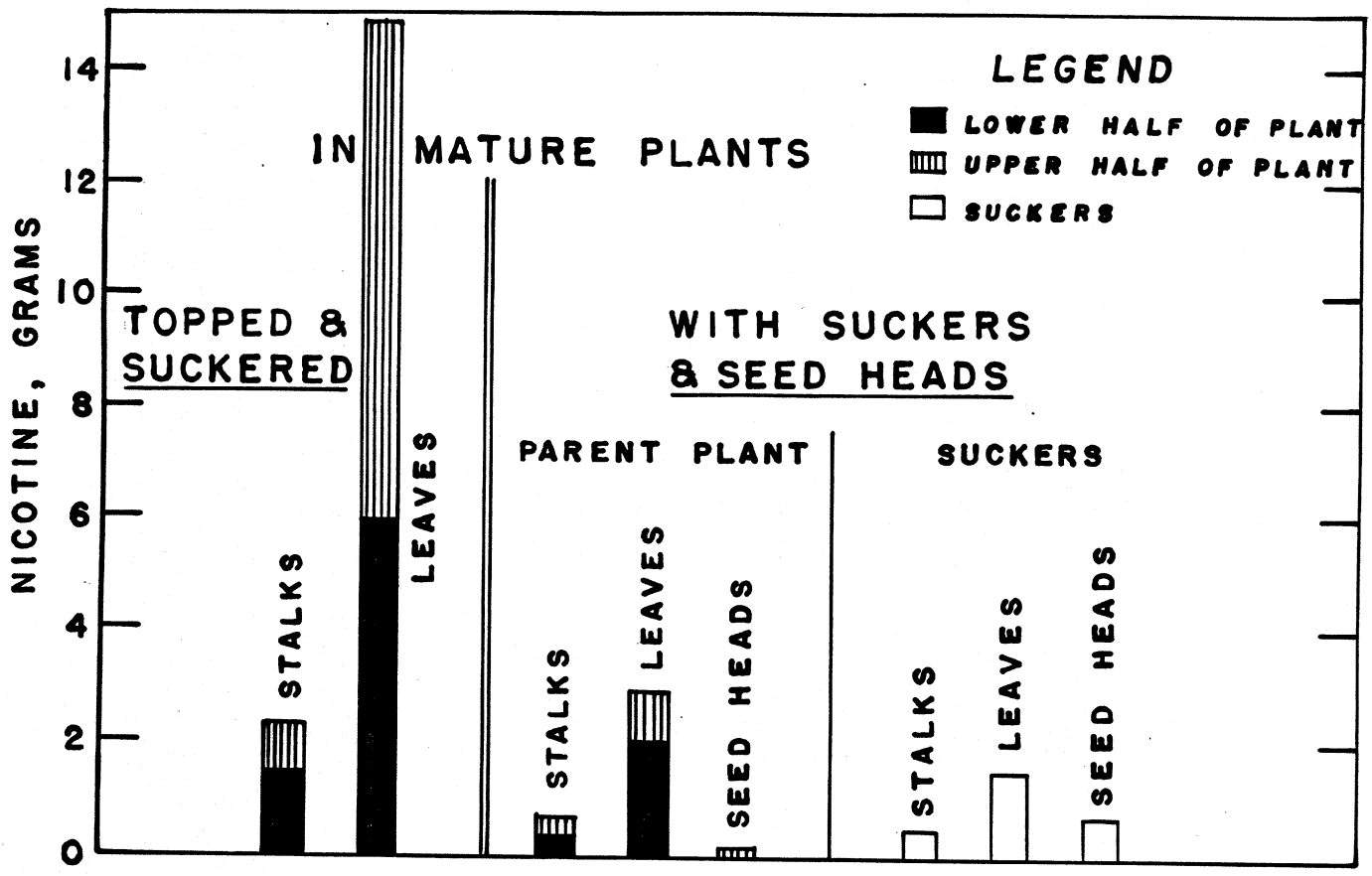
The leaves contained 86.26 percent of the total nicotine of the plant; 94.4 percent of the leaf nicotine was in the leaf blades.

Although the root section was included in this study, the data in the table are included to show a trend only. Care was taken to include all the root system, but there was no assurance that the weight given in the table was correct because the spongelike form of the roots made it difficult to remove all the soil and to completely remove the surface water left after washing.

Table 11. Distribution of weight and of nicotine in *N. rustica*, variety 23, Plant A. Planted June 18, 1946. Tops and suckers removed August 14 and August 30. Harvested September 10, 84 days after setting. Nicotine content: 17.14 grams (minus nicotine in roots); 17.57 grams (including nicotine in roots).

Green plant	% of total weight	Moisture %	Nicotine in fraction %[1]	% of total Nicotine[2]		
Whole plant	100.0	86.4[3]	5.26[3]			
Plant minus roots	92.4	87.5[3]	6.02[3]	100.00		
Stalk	29.0	84.5[3]	2.13[3]	13.74		
Lower half stalk	18.8	82.4	1.82			8.65
Upper half stalk	10.1	88.3	2.99			5.08
Leaves	63.4	88.8[3]	8.48[3]	86.26		
Lower half leaves	31.8	90.3[3]	7.83[3]		34.64	
Lower half midribs and petioles	6.9	91.0	2.10			1.88
Lower half leaf blades	24.9	90.1	9.28			32.76
Upper half leaves	31.6	87.4[3]	8.99[3]		51.62	
Upper half midribs and petioles	7.6	88.8	2.41			2.93
Upper half leaf blades	24.1	86.9	10.76			48.69
Roots	7.6	73.6	0.87		---	

[1] Moisture-free basis.
[2] Not including nicotine in roots.
[3] Calculated value.


NICOTINE, GRAMS
0
2
4
6
8
10
12
14
IN MATURE PLANTS
LEGEND
LOWER HALF OF PLANT
UPPER HALF OF PLANT
SUCKERS
TOPPED & SUCKERED
STALKS
LEAVES
WITH SUCKERS & SEED HEADS
PARENT PLANT
STALKS
LEAVES
SEED HEADS
SUCKERS
STALKS
LEAVES
SEED HEADS

In table 12 (plant B), a larger number of plant divisions were taken because the suckers and seed heads were retained on this plant. The three main parts studied were the suckers, the remainder of the plant, and the roots. The root section was treated separately as in table 11. Each of the two main sections were divided into stalk, leaves, and seed heads. After removal of the suckers, the leaves and stalk of the plant were divided into the same subsections as described for plant A, table 11.

(Table 12)

Table 12 shows that the main part of the plant (plant minus suckers and roots) accounted for 51.6 percent and the suckers 44.5 percent of the total weight of the green plant. The combined seed heads of the main plant and the suckers accounted for 21.7 percent of the weight. The moisture of the different segments was fairly uniform, ranging from 83.1 to 92.3 percent. The segments with the highest percentage of nicotine were the upper and lower leaf blades of the main plant, they had 4.12 and 4.41 percent nicotine, respectively, nearly 1 percent higher than the leaf blade section of the suckers. The seed heads, the midribs and petioles, and the stalks had a nicotine content of approximately 1 percent.

The data show that 59.27 percent of the total nicotine occurred in the main plant and 40.73 percent in the suckers. As was true of the topped and suckered plant, the greatest proportion of the nicotine was present in the leaves, which had 70.8 percent of the total nicotine. This percentage of nicotine is considerably less than the 86.26 percent nicotine found in the leaves of the topped and suckered plant. This lower nicotine value is undoubtedly related to the seed head of the unpruned plant. Figures 6, 7, and 8 show graphically the distribution of nicotine in the plants studied.

Although the two plants were of approximately equal age and weight, the amount of nicotine produced varied considerably. The unpruned plant (B) produced only 6.14 grams of nicotine, whereas the topped and suckered plant (A) produced almost three times as much, 17.14 grams. This is in accord with the findings of McMurtrey et al. (2).

Leaf Areas

The number of leaves normal to this variety ranges from 22 to 28. The leaves are ovate and crinkled, and mature in 85 to 90 days under normal growing conditions. Fifty-seven days after setting, plants A and B were selected, since they were judged to be typical and equal in size and in leaf area. The difference in growth of the two plants from the time of selection until the date of harvest is well demonstrated by the difference in leaf measurements (table 13).

(Table 13)

Accurate measurement of the area of a crinkled *N. rustica* leaf is difficult and involves a large factor of error. The table shows that the leaves of the upper half of the pruned plant A had larger areas than the leaves of the

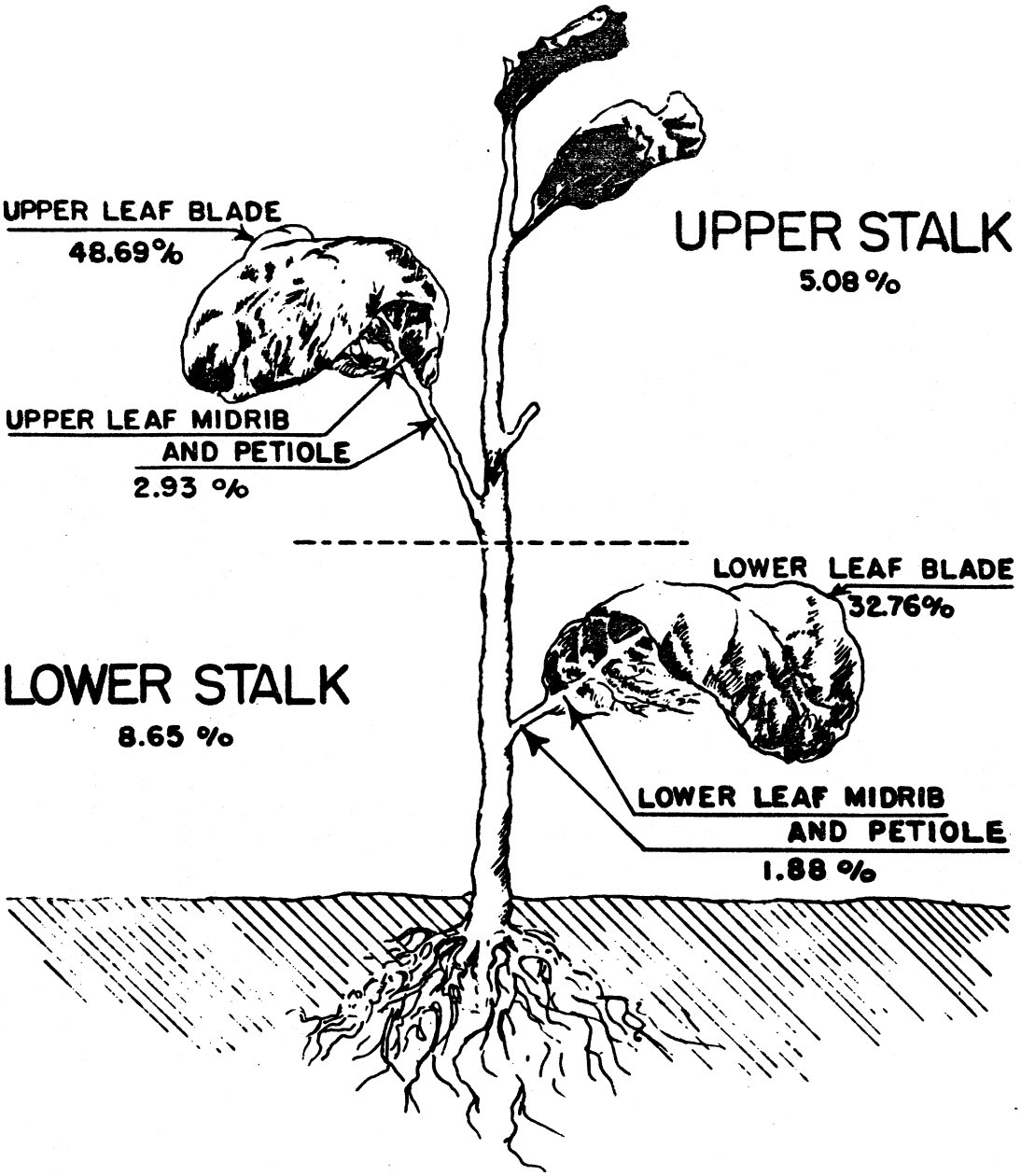


Figure 7.—Distribution of nicotine in a N. rustica plant from which tops and suckers had been removed.

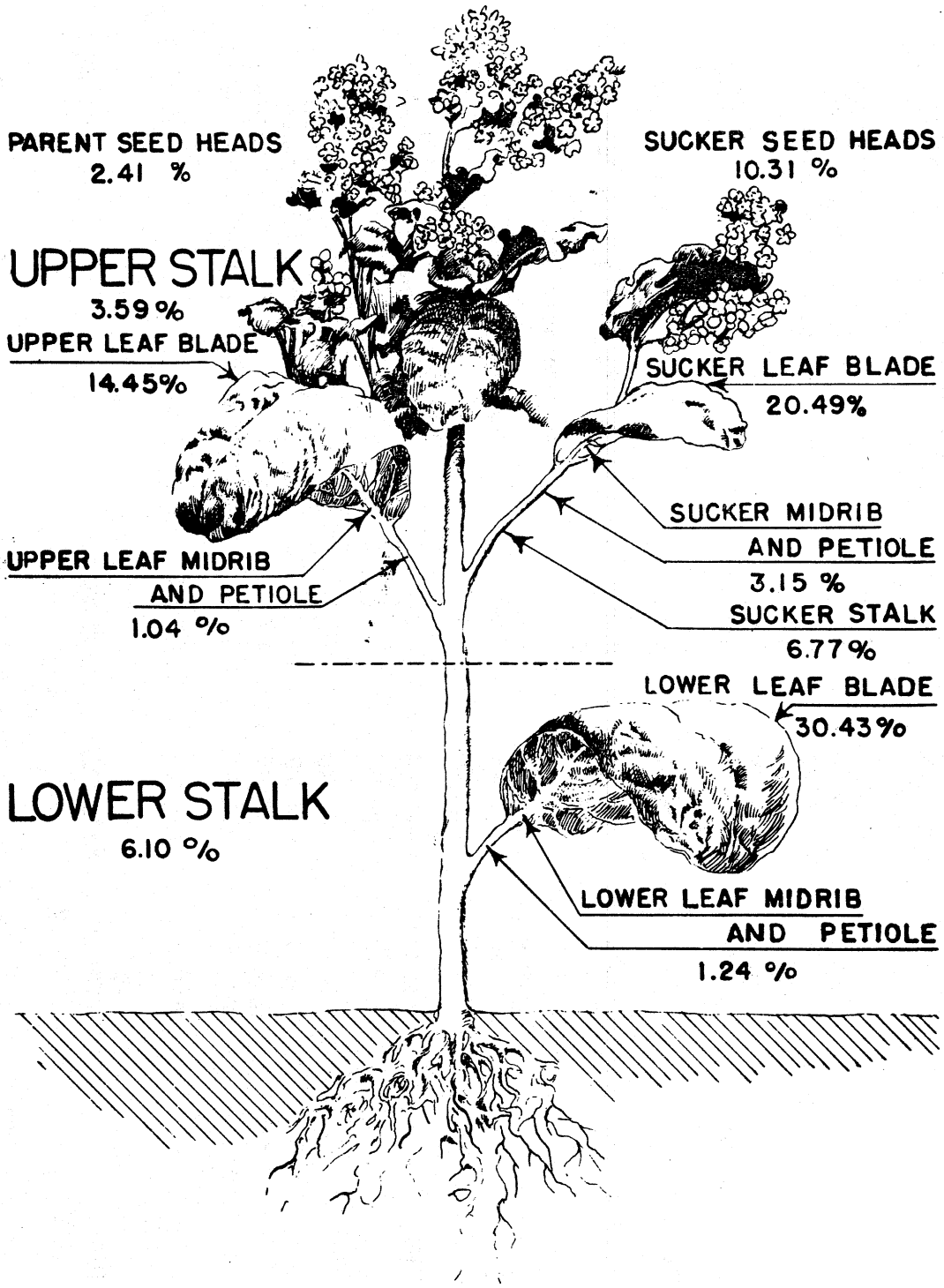

PARENT SEED HEADS
2.41 %
SUCKER SEED HEADS
10.31 %
UPPER STALK
3.59 %
UPPER LEAF BLADE
14.45%
SUCKER LEAF BLADE
20.49%
SUCKER MIDRIB
AND PETIOLE
3.15 %
UPPER LEAF MIDRIB
AND PETIOLE
1.04 %
SUCKER STALK
6.77%
LOWER LEAF BLADE
30.43%
LOWER STALK
6.10 %
LOWER LEAF MIDRIB
AND PETIOLE
1.24 %

Table 12. Distribution of weight and nicotine in *N. rustica*, variety 23, Plant B. Tops and suckers not removed. Planted June 18, 1946. Harvested Sept. 5, 79 days after setting. Nicotine content: 6.14 grams (minus nicotine in roots); 6.65 grams (including nicotine in roots)

Plant part	% of total weight	Moisture %	Nicotine in fraction %[1]		% of total nicotine[2]	
Whole plant	100.0	88.1[3]	1.97[3]		---	
Plant minus suckers and roots	51.6	87.7[3]	2.02[3]	59.27		
Stalk	18.5	85.4[3]	0.77[3]		9.69	
Lower half stalk	12.0	83.1	0.65			6.10
Upper half stalk	6.5	89.5	1.14			3.59
Leaves	27.3	89.7[3]	3.64[3]			
Lower half leaves	18.4	90.0[3]	3.73[3]		31.68	
Lower half midribs and petioles	4.5	92.3	0.78			1.24
Lower half leaf blades	13.9	89.3	4.41			30.44
Upper half leaves	8.9	89.2[3]	3.48[3]		15.49	
Upper half midribs and petioles	2.6	92.2	1.15			1.04
Upper half leaf blades	6.3	87.9	4.12			14.45
Seed heads	5.8	85.8	0.63		2.41	
Suckers	44.5	88.4[3]	1.70[3]	40.73		
Stalk	13.3	89.7	1.07		6.77	
Leaves	15.4	88.7[3]	2.93[3]		23.65	
Midribs and petioles	4.3	92.0	2.00			3.16
Leaf blade	11.1	87.4	3.16			20.49
Seed heads	15.9	87.0	1.08		10.31	
Roots	3.9	89.7	4.47			
Plants with suckers (not including roots)	96.1	88.0[3]	1.88[3]	100.00		

1/ Moisture-free basis.
2/ Not including nicotine in roots.
3/ Calculated values.

Table 13. Dimensions of leaves of plants A and B

	Average length	Average width	Average area	Total area
	cm.	cm.	cm^2	cm^2
Plant A (tops and suckers removed)				
Leaves (upper half)	32	24	8004	
Leaves (lower half)	30	25	7872	15,876
Plant B (tops and suckers not removed)				
Leaves (upper half)	21	17	3552	
Leaves (lower half)	28	23	6516	
Leaves (sucker)	-	-	6189[1/]	16,257

1/ Estimated.

intact plant B (8000 cm^2 : 3500 cm^2). This is further shown by the greater length and width of the leaves of the pruned plant.

The lower leaves showed a similar difference in dimensions but of less degree (7900 cm^2 for plant A and 6500 cm^2 for plant B).

GROWTH OF *NICOTIANA RUSTICA* AT WYNDMOOR, PA.

E. G. Beinhart

Although *Nicotiana rustica* was grown here to supply material for the technical studies described, some incidental observations were made on the behavior of the crop. In general it grew well, producing large, vigorous plants (figures 4 and 5). Production of 2000 pounds of leaves (table 2) and from 100 to 200 pounds of nicotine per acre (tables 1, 2 and 3) compares favorably with the best reported by McMurtrey et al. (2), as does also the percentage of nicotine in the leaf (7 to 9 percent in general).

The data in tables 4, 6, and 8 indicate that the percentage of nicotine in the leaf reached a peak in general about 90 to 100 days after transplanting.

Summary

Although the main purpose of this three-season study was to determine whether nicotine is lost during the drying of *Nicotiana rustica* and, if so, under what conditions, other objectives were the development of adequate sampling methods and determination of distribution of nicotine in various parts of the plant.

Nicotine was determined in 1946 and 1947 by the A.O.A.C. silicotungstic acid method, and in 1948 by the spectrophotometric method. Use of the modified Griffith-Jeffrey still greatly speeded the operations. Statistical analyses of the nicotine data showed that the new sampling method was adequate.

The high moisture content of fresh tobacco leaves, and the ease with which moisture is lost from cut plants, made sampling one of the most difficult problems. The moisture and nicotine determinations had to have a high degree of precision and accuracy in order that small nicotine changes might be correlated with drying conditions. A satisfactory method that gave the desired results was eventually developed. The cut leaves were mixed in a closed container, after which all samples were removed simultaneously. For moisture determination, the sample was dried at 65° C. to 12 to 15 percent moisture, ground, subsampled, and dried to constant weight at 110°.

Each year part of the crop was dried in an unheated barn by hanging the whole plant on sticks. The leaves dried in 4 to 6 weeks; the stalks dried in 13 to 17 weeks. There was no evidence of loss of nicotine during this drying, or of translocation of nicotine from the leaves to the stalks.

In the oven-drying experiments in 1946, there appeared to be no significant loss of nicotine when the residual moisture was 12 to 15 percent. When the moisture was 3 to 5 percent, the loss was significant. The 1947 data, however, showed a significant relation between loss of nicotine and the maximum

drying temperature; the average loss at temperatures of 108° to 116° C. was 6.3 percent. The data obtained in 1948 confirmed this relation.

To study this matter further, air at 140° C. was passed through ground leaves in a closed tube, and the residual material and the products in the air stream were analyzed for nicotine. Loss of nicotine from the leaves was immediate, and continued for at least 6 hours, by which time more than 50 percent of the nicotine had been lost. About 17 percent was recovered as nicotine in the distillate; the rest represented chemical destruction of the nicotine.

Two plants, one intact and the other kept pruned of seed head and suckers during growth, were separated into the various parts, and each part was analyzed for nicotine. Approximate percentages of nicotine in the dry matter of the intact plant were: whole plant, 2.0; stalk, 0.8; leaf blades, 4.3; midribs and petioles, 1.0; seed heads, 0.6; sucker leaf blades, 3.2; roots, 4.5. The figures for the pruned plant were: whole plant, 5.3; stalk, 2.1; leaf blades, 10.0; midribs and petioles, 2.3; roots, 0.9. The leaves of the intact plant contained 69 percent of the total nicotine, and those of the pruned plant contained 86 percent.

Nicotiana rustica grew well at Wyndmoor, Pa., producing up to 2000 pounds of dry leaves and 200 pounds of nicotine per acre.

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